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ERGOT ALKALOIDS

XXXVI*. SEPARATION OF DIASTEREOMERIC (+)-1-HYDROXY-2-BUTYLAMIDES OF D- AND L-LYSERGIC AND D- AND L-ISOLYSERGIC ACIDS BY THIN-LAYER CHROMATOGRAPHY

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SUMMARY

A procedure for the separation of diastereomeric (+)-1-hydroxy-2-butylamides of D- and L-lysergic and D- and L-isolysergic acids by thin-layer chromatography has been developed. By using aqueous solutions of imidazole as one of the components of the solvent system, greater differences were found in the R_F values of the substances studied than was the case without imidazole.

INTRODUCTION

The object of this study was to find conditions giving a good separation of diastereomeric (+)-1-hydroxy-2-butylamides of D- and L-lysergic and D- and L-isolysergic acids (I–IV) on thin layers of silica gel and alumina, and to attempt to use the solubilization effect of imidazole for the purpose. The idea of using imidazole originated after we had found experimentally, in the course of our preparatory work, that imidazole could solubilize methylergobasine (I) and methylergobasinine (III) in aqueous medium.

MACEK AND VANĚČEK[1] have studied the separation of substances I–IV by paper chromatography. However, the differences in the R_F values of the amides I and II were too slight. Various authors have used thin-layer chromatography for the separation of amides of lysergic acid (see refs. 2–7). For instance, GENEST AND FARMILO[4,5] have separated the diethylamide of lysergic acid alongside of heroin and other narcotics. GENEST[5] determined the diethylamide of lysergic acid so isolated

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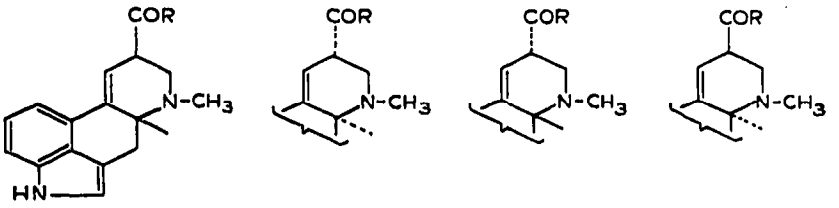

I II III IV

I – IV R = NH CH(CH_2OH) CH_2CH_3-(+)

by a densitometric method. BIANCHINE *et al.*[3] used this procedure for the separation of methysergid, *i.e.* (+)-1-hydroxy-2-butylamide of 1-methyl-D-lysergic acid, and methergin (I) in a pure form and after extraction from biological tissues and fluids. HOFMANN AND TSCHERTER[6,7] determined the amides of lysergic and isolysergic acids, chamoclavine, elymoclavine and lysergol in *Rives corymbosa* and *Ipomoea tricolor* Cava by thin-layer chromatography.

EXPERIMENTAL

Chemicals and reagents

The amides I–IV used were prepared in our laboratories[8]. All the substances were chromatographically pure. Chemicals were of reagent grade quality. Imidazole pur. was manufactured by Messrs. Koch-Light. Chromatographic separation was carried out on Silica Gel G Merck layers with the addition of 2% of a fluorescence indicator (UV 254 nm). Aluminium oxide, Lachema, Czechoslovak Standard 685131, pH 8.6, grain size 0.075 mm, was also used, activity approx. III (azobenzene R_F 0.70; *p*-methoxyazobenzene 0.42; Sudan Yellow 0.20; Sudan Red 0.06 and *p*-aminoazobenzene 0.03, in carbon tetrachloride).

Apparatus

The usual type of equipment for chromatographic separation[9,10] was used. Substances isolated were detected by means of a Uvis-lamp (Desaga) at wavelengths of 254 and 365 nm.

Counter-current distribution was studied in a device consisting of six ground-glass separators on a common axis[11]. A Unicam IR-700 spectrophotometer was used.

Method

After preparation, the layers of Silica Gel G were dried for 24 h at room temperature. Prior to use the layers were heated at 100–110° for 30 min in an oven.

Layers of aluminium oxide were activated and adjusted in the usual way[10]. Separated substances and their mixtures were spotted as a 0.05% solution in 60% ethanol in amounts of 10 μ on the chromatographic plates (dimensions 10 × 24 cm). Chromatographic separation was performed in chambers which were saturated for 30 min with the solvent system used in the case of silica gel, while the saturation time was 120 min when loose aluminium oxide was used.

The partition coefficient K was determined by shaking 4 ml of a solution of

amide I or amide III in ether–ethanol (95 : 5) (concentration 1 mg of base in 1 ml of the solution) with 4 ml of water, or with 4 ml of a 0.01, 0.1, 1 or 2 *M* aqueous solution of imidazole. The concentration of the amides was determined spectrophotometrically at 315 nm; $K = C_1/C_2$ (C_1 = concentration of amides in organic phase, C_2 = concentration of amides in aqueous phase).

RESULTS AND DISCUSSION

In experiments described above the solubilization effect of the imidazole upon amides I and III was verified by the determination of their partition coefficient K in the systems ether–ethanol (95 : 5) / water and ether–ethanol (95 : 5) / aqueous solution of imidazole. In the first system, K for amide I was 0.94 and K for amide III was 7.51, in the second system, using 1 *M* and 2 *M* imidazole, substantially lower values were found as a result of solubilization ($K = 0.2$ for amide I and $K = 1.26$ for amide III with 2 *M* imidazole); the solubilization effect of 0.01 *M* and 0.1 *M* imidazole was only slight and the partition coefficients were nearly the same as when water was used in the first system.

The results of our experiments for the separation of amides I–IV on thin layers of silica gel and alumina, in various solvent systems, and also the effect of imidazole on the separation of these amides are summarized in Table I.

As was the case in paper chromatography, the R_F values of amides III and IV

TABLE I

SEPARATION OF DIASTEREOMERIC (+)-1-HYDROXY-2-BUTYLAMIDES I–IV BY THIN-LAYER CHROMATOGRAPHY

Number of solvent system	*Solvent system*	*R_F values*							
		Silica gel				*Aluminum oxide*			
		I	*II*	*III*	*IV*	*I*	*II*	*III*	*IV*
1	Ether–ethanol (95:5)	0.07	0.07	0.07	0.07	0.60	0.60	0.75	0.75
2	Ether–ethanol–water (90:5:5)	0.35	0.15	0.47	0.68				
3	Ether–ethanol–0.1 *M* imidazole (90:5:5)	0.28	0.08	0.35	0.58				
4	Ether–ethanol–0.75 *M* imidazole (90:5:5)	0.30	0.13	0.54	0.69				
5	Ether–ethanol–1 *M* imidazole (90:5:5)	0.48	0.35	0.65	0.75	0.76	0.76	0.76	0.76
6	Chloroform–acetone (50:50)	0.20	0.20	0.25	0.25	0.47	0.47	0.75	0.75
7	Chloroform–acetone–water (50:45:5)	0.12	0.07	0.27	0.35				
8	Chloroform–acetone–0.1 *M* imidazole (50:45:5)	0.22	0.15	0.51	0.75	0.57	0.57	0.75	0.75
9	Chloroform–acetone–1 *M* imidazole (50:45:5)	0.27	0.07	0.31	0.47	0.50	0.32	0.62	0.70
10	Chloroform–ethanol (90:10)	0.72	0.60	0.80	0.80	0.87	0.87	0.93	0.93
11	Chloroform–ethanol–water (90:5:5)	0.30	0.27	0.43	0.50				
12	Chloroform–ethanol–0.1 *M* imidazole (90:5:5)	0.42	0.35	0.62	0.82	0.55	0.47	0.78	0.80
13	Chloroform–ethanol–1 *M* imidazole (90:5:5)	0.55	0.50	0.65	0.80	0.68	0.84	0.92	0.96

TABLE II

VISCOSITY AND pH DATA OF IMIDAZOLE AQUEOUS SOLUTIONS

Molarity of imidazole solution	*Viscosity**		*pH*
	Absolute	*Kinematic in cSt*	
0.1	1.1323	1.1323	9.65
0.2	1.1575	1.1575	9.55
0.5	1.1862	1.1840	9.40
0.75	1.2831	1.2778	9.30
1.0	1.3334	1.3250	9.2

* Wt. of ball used: 4.9789 g; specific weight at 20°: 2.409 g; ball constant: 0.009987.

were higher on thin layers as compared with amides I and II. Solvent systems including water (No. 2, 7, 11) and analogous systems using aqueous solutions of imidazole (systems No. 3, 4, 5, 8, 9, 12, 13) were shown to be useful for the separation of all four stereoisomeric amides I–IV. The effect of imidazole upon the separation of the compounds under study was followed for system No. 2 by using 0.1, 0.2, 0.3, 0.4, 0.5, 0.75, 0.8 and 1.0 *M* aqueous solutions of imidazole instead of water. By using the system ether–ethanol–0.75 *M* imidazole (No. 4) the difference between the R_F values of substances II and III was greater than that in system No. 2. Comparison of the solvent systems chloroform–acetone–water (No. 7) and chloroform–acetone–0.1 *M* imidazole (No. 8) shows the favorable effect of imidazole on the separation of compounds III and IV. The presence of imidazole in the solvent systems made it possible, in contrast to systems without imidazole, to obtain compact and clearly outlined spots of I–IV. The separation of the substances studied did not directly depend on the basicity of the used system (see Table II).

The favorable effect of imidazole on the separation of the amides I–IV, up to a certain concentration of the added solubilizer, can be explained by formation of intermolecular bonds between this component of the solvent system with the butanolamides present. A concentration of 0.75 *M* imidazole was found experimentally to be the most favorable for the separation. In concentrations above this limit, the differences in the R_F values of the substances I–IV separated were decreased and the separation coefficients also increased. Thus, in excessively concentrated solutions the solubilizing effect decreases and hence the separation of these substances on thin layers is worse. This phenomenon is in accordance with the data of HECKER[13], GENEST[5] and KÜTTEL[14].

When the solvent systems are modified as in this study, not only does a change occur in the R_F values on thin-layer chromatography of the substances studied at higher concentrations of added imidazole, but a considerable increase of viscosity was found experimentally (Table II), which is in agreement with BÜCHI[15], who studied this problem by measuring the surface tension of the solubilizers.

Attempts to interpret the data obtained by IR spectroscopy and NMR measurements with a view to proving a direct or indirect interaction of imidazole with the diasteroisomeric amides I–IV has not met with any success so far. The solution of this problem will be the subject of further study.

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